

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for producing an oligosaccharide comprising lactose ~~by using~~ a genetically modified cell starting with at least one internalized exogenous precursor ~~selected from the group consisting of lactose, sialic acid, α -galactoside, and β -galactoside;~~ said method comprising:

- (i) obtaining an Z⁻Y⁺ E. coli cell that comprises at least one recombinant gene encoding an enzyme capable of modifying said exogenous precursor or one of the intermediates in the biosynthetic pathway of said oligosaccharide from said exogenous precursor necessary for the synthesis of said oligosaccharide from said exogenous precursor, and also the components for expressing said gene in said cell;

~~lacks any enzymatic activity liable to degrade said oligosaccharide, said precursor and said intermediates; and~~

- (ii) culturing said cell on a carbon-based substrate in the presence of at least one said exogenous precursor, under conditions inducing the internalization according to a mechanism of active transport of said exogenous precursor by said cell and the production of said oligosaccharide by said cell,

wherein said culturing comprises:

(a) a first phase of exponential cell growth ensured by said carbon-based substrate,

(b) a second phase of cell growth limited by said carbon-based substrate which is added continuously,

wherein said precursor is added during the second phase.

2.-4. (Canceled)

5. (Previously Presented) The method as claimed in claim 1, wherein said modification is selected from the group consisting of glycosylation, sulfatation, acetylation, phosphorylation, succinylation, methylation, and addition of an enolpyruvate group.

6. (Previously Presented) The method as claimed in claim 1, wherein said enzyme is an enzyme capable of performing a glycosylation, chosen from glycosyl-transferases.

7. (Previously Presented) The method as claimed in claim 6, wherein said enzyme is a glycosyl-transferase selected from the group consisting of β -1,3-N-acetyl-glucosaminyl-transferase, β -1, 3-galactosyl-transferase, β -1, 3-N-acetyl-galactosaminyl-transferase, β -1, 3-glucuronosyl-transferase, β -1, 3-N-acetyl-galactosaminyl-transferase, β -1, 4-N-acetyl-galactosaminyl-transferase, β -1,4-galactosyl-transferase, α -1,3-galactosyl-transferase, α -1, 4-galactosyl-transferase, α -2, 3-sialyl-transferase, α -2, 6-sialyl-transferase, α -2, 8-sialyl-transferase, α -1, 2-fucosyl-transferase, α -1, 3-fucosyl-transferase and α -1, 4-fucosyl-transferase.

8. (Canceled).

9. (Currently Amended) The method as claimed in claim 8~~1~~, wherein said carbon-based substrate is selected from the group consisting of glycerol and glucose.

10. (Currently Amended) The method as claimed in claim 8~~1~~, wherein said culturing is performed under conditions allowing the production of a culture with a high cell density.

11. (Currently Amended) The method as claimed in claim 10, wherein said culturing ~~step~~ further comprises:

~~—— a) a first phase of exponential cell growth ensured by said carbon-based substrate;~~

~~—— b) a second phase of cell growth limited by said carbon-based substrate which is added continuously;~~

(c) a third phase of slowed cell growth obtained by continuously adding to the culture an amount of said substrate that is less than the amount of substrate added in ~~step b)~~ said second phase so as to increase the content of oligosaccharides produced in the high cell density culture.

12. (Currently Amended) The method as claimed in claim 11, wherein the amount of substrate added continuously to the cell culture during said third phase-e) is at least 30% less than the amount of substrate added continuously during said second phase-b).

13. (Canceled).

14. (Canceled).

15. (Withdrawn) The method as claimed in claim 1, wherein said precursor is a monosaccharide whose anomeric carbon is linked to an alkyl group so as to allow its internalization by a mechanism of passive transport.

16. (Withdrawn) The method as claimed in claim 15, wherein said alkyl group is an allyl.

17. (Withdrawn) The method as claimed in claim 15, for the production of (β -D-Gal-[1 \rightarrow 4]- β -D-GlcNac-1 \rightarrow O-allyl), wherein

- said cell is a bacterium of *LacZ* genotype;
- said enzyme is β -1, 4-galactosyl-transferase;
- said substrate is glycerol;
- said precursor is allyl-N-acetyl- β -D-glucosaminide (β -D-GlcNac-1 \rightarrow O-allyl).

18.-20. (Canceled).

21. (Withdrawn) The method as claimed in claim 1, wherein said precursor is sialic acid.

22. (Withdrawn) The method as claimed in claim 21, wherein said active transport of said precursor is performed by NanT permease.

23. (Withdrawn) The method as claimed in claim 1, wherein said precursor is sialic acid and lactose.

24. (Withdrawn) The method as claimed in claim 23, wherein said active transport of said precursor is performed by lactose permease and NanT permease.

25. (Canceled).

26. (Canceled).

27. (Previously Presented) The method as claimed in claim 1, further comprising the addition of an inducer to said culture medium to induce the expression in said cell of said enzyme and/or of a protein involved in said transport.

28. (Previously Presented) The method as claimed in claim 27, wherein said inducer is isopropyl β -D-thiogalactoside (IPTG) and said protein is lactose permease.

29. (Withdrawn) The method as claimed in claim 1, for the production of the trisaccharide 4-O-[3-O- (2-acetamido-2-deoxy- β -D-glucopyranosyl) - β -D-galactopyranosyl] -D-glucopyranose, (β -D-GlcNac-[1 \rightarrow 3] - β -D-Gal-[1 \rightarrow 4] -D-Glc), wherein:

- said cell is a bacterium of *LacZ*, *LacY*⁺ genotype;
- said enzyme is β -1, 3-N-acetyl-glucosaminyl-transferase;
- said substrate is glycerol;
- said inducer is isopropyl β -D-thiogalactoside (IPTG); and
- said precursor is lactose.

30. (Previously Presented) The method as claimed in claim 1, for the production of lacto-N-neo-tetraose and polylactosamine (lacto-N-neo-hexaose, lacto-N-neo-octaose, lacto-N-neo-decaose), further comprising the addition of an inducer to said culture medium to induce the expression in said cell of said enzyme and/or of a protein involved in said transport-wherein:

- said cell is a bacterium of *LacZ*, *LacY*⁺ genotype;
- said enzymes are β -1, 3-N-acetyl-glucosaminyl-transferase and β -1, 4-galactosyl-transferase;
- said inducer is isopropyl- β -D-thiogalactoside (IPTG); and
- said precursor is lactose.

31. (Withdrawn) The method as claimed in claim 30, for the production of a sialyl derivative of lacto-N-neo-tetraose and of polylactosamine (lacto-N-neo-hexaose, lacto-

N-neo-octaose, lacto-N-neo-decaose), further comprising an enzyme chosen from α -2, 3-sialyl-transferase and α -2, 6-sialyl-transferase, and wherein said cell has a *NanA*⁻, *NanT*⁺ genotype and expresses the gene for CMP-NeuAc-synthase.

32. (Withdrawn) The method as claimed in claim 30, for the production of a fucosyl derivative of lacto-N-neo-tetraose and of polylectosamine (lacto-N-neo-hexaose, lacto-N-neo-octaose, lacto-N-neo-decaose), further comprising an enzyme chosen from α -1, 2-fucosyl-transferase and α -1, 3-fucosyl-transferase, and wherein said cell has a *WcaJ*⁻ genotype and overexpresses the *RcsA* gene.

33. (Withdrawn) The method as claimed in claim 30, for the production of a sialyl and fucosyl derivative of lacto-N-neo-tetraose, lacto-N-neo-decaose, further comprising an enzyme chosen from α -2,3-sialyl-transferase and α -2,6-sialyl-transferase, and an enzyme chosen from α -1,2-fucosyl-transferase and α -1,3-fucosyl-transferase, and wherein said cell has a *NanA*⁻, *NanT*⁺, *WcaJ* genotype and overexpresses the *RcsA* gene and the gene for CMP-NeuAc-synthase.

34. (Withdrawn) The method as claimed in claim 1, for the production of 3'-sialyllactose (α -NeuAc-[2→3] - β -D-Gal-[1→4] - β -D-Glc) or 6'-sialyllactose (α -NeuAc-[2→6] - β -D-Gal-[1→4] - β -D-Glc), wherein:

- said cell is a bacterium of *LacZ*⁻, *LacY*⁺, *NanA* or *NanT*⁺ genotype;
- said enzymes are CMP-NeuAc-synthase and α -2, 3-sialyl-transferase or α -2, 6-sialyl-transferase;
- said substrate is glycerol;
- said inducer is isopropyl- β -D-thiogalactoside (IPTG); and
- said precursors are lactose and sialic acid.

35. (Withdrawn) The method as claimed in claim 1, for the production of 3'-fucosyllactose (β -D-Gal-[1→4]-(α -L-Fuc-[1→3]-D-Glc) or 2'-fucosyllactose, α -L-Fuc-[1→2] - β -D-Gal- [1→4] -D-Glc further comprising an enzyme chosen from α -1, 3-fucosyl-transferase or α -1, 2-fucosyl-transferase, and wherein said cell has a *wcaJ*⁻ *lacZ* genotype and overexpresses the *rcaA* gene and wherein said precursor is lactose.

36. (Withdrawn) The method as claimed in claim 1, for the production of allyl 3-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)- β -D-galactopyranoside, (β -D-GlcNac-[1 \rightarrow 3]- β -D-Gal-1 \rightarrow O-allyl), wherein:

- said cell is a bacterium of *LacZ*, *LacY*⁺ genotype;
- said enzyme is β -1, 3-N-acetyl-glucosaminyl-transferase;
- said substrate is glycerol;
- said inducer is isopropyl β -D-thiogalactoside (IPTG); and
- said precursor is allyl- β -D-galactopyranoside.

37. (Withdrawn) The method as claimed in claim 1, for the production of analogs of lacto-N-neo-tetraose and of polylactosamines in which the glucose residue is replaced with an allyl group, c wherein

- said cell is a bacterium of *LacZ*, *LacY*⁺ genotype;
- said enzymes are β -1,3-N-acetyl-glucosaminyl-transferase and β -1,4-galactosyl-transferase;
- said substrate is glucose;
- said inducer is isopropyl β -D-thiogalactoside (IPTG); and
- said precursor is allyl- β -D-galactopyranoside.

38. (Withdrawn) The method as claimed in claim 31, for the production of oligosaccharide analogs in which the glucose residue is replaced with an allyl group, wherein said precursor is allyl- β -D-galactoside.

39. (Currently Amended) The method as claimed in claim 1, ~~for producing an oligosaccharide labeled with at least one isotope~~, wherein said cell is cultured on said carbon-based substrate labeled with said at least one isotope ~~and/or~~ in the presence of said exogenous precursor labeled with said isotope.

40. (Withdrawn) An oligosaccharide which may be obtained by the method as claimed in claim 1.

41. (Withdrawn) An oligosaccharide which may be obtained by the method as claimed in claim 17, characterized in that the double bond of the allyl group of said oligosaccharides is chemically modified by addition, oxidation or ozonolysis reactions to form activated oligosaccharides that may be used for the chemical synthesis of glycoconjugates or glycopolymers.

42. (Withdrawn) The oligosaccharide as claimed in claim 40, as a medicinal product.

43. (Withdrawn) The oligosaccharide as claimed in claim 42, as a medicinal product intended to selectively prevent the adhesion of biological molecules.

44. (Withdrawn) The oligosaccharide as claimed in claim 42, as a medicinal product intended for treating cancer, inflammation, heart diseases, diabetes, bacterial infections, viral infections and neurological diseases and grafts.

45. (Withdrawn) A pharmaceutical composition, characterized in that it comprises an oligosaccharide as claimed in claim 42 and a pharmaceutically acceptable vehicle.

46. (Withdrawn) The agricultural or agronomic use of an oligosaccharide as claimed in claim 40, especially for the growth and defense of plants.

47. (Currently Amended) A method for modifying an exogenous precursor selected from the group consisting of lactose, sialic acid, α -galactoside, and β -galactoside useful in the synthesis of an oligosaccharide ~~selected from the group consisting of lactose, sialic acid, α -galactoside, and β -galactoside~~ using a genetically modified cell comprising:

- (i) obtaining an *E. coli* cell having a *LacZ*, *LacY*⁺, *NanA* or *NanT*⁺ genotype, wherein said cell ~~comprising~~ comprises (a) at least one recombinant gene encoding an enzyme capable of modifying said exogenous precursor, and (b) the components for expressing said gene in said cell, ~~wherein said cell lacks any enzymatic activity liable to degrade said oligosaccharide, said precursor and said intermediates;~~

- (ii) culturing said cell on a carbon-based substrate in the presence of at least one said exogenous precursor under conditions sufficient to cause expression of said recombinant gene; and
- (iii) exposing said cell to lactose permease under conditions sufficient to induce the internalization of said exogenous precursor by said cell,

wherein the enzyme is a glycosyl-transferase selected from the group consisting of β -1,3-N-acetyl-glucosaminyl-transferase, β -1, 3-galactosyl-transferase, β -1, 3-N-acetyl-galactosaminyl-transferase, β -1, 3-glucuronosyl-transferase, β -1, 3-N-acetyl-galactosaminyl-transferase, β -1, 4-N-acetyl-galactosaminyl-transferase, β -1,4-galactosyl-transferase, α -1,3-galactosyl-transferase, α -1, 4-galactosyl-transferase, α -2, 3-sialyl-transferase, α -2, 6-sialyl-transferase, α -2, 8-sialyl-transferase, α -1, 2-fucosyl-transferase, α -1, 3-fucosyl-transferase and α -1, 4-fucosyl-transferase, and
wherein said culturing comprises:

(a) a first phase of exponential cell growth ensured by said carbon-based substrate;

(b) a second phase of cell growth limited by said carbon-based substrate which is added continuously; and
wherein said precursor is added during the second phase.

48. (Currently Amended) A method for producing an oligosaccharide comprising lactose by a genetically modified cell starting with at least one internalized exogenous precursor ~~selected from the group~~ consisting of lactose, ~~sialic acid, α -galactoside, and β -galactoside,~~ said method comprising:

- (i) obtaining a an Z⁻Y⁺ *E. coli* cell comprising (a) at least one recombinant gene encoding an enzyme capable of modifying said exogenous precursor, and (b) the components for expressing said gene in said cell, ~~wherein said cell lacks any enzymatic activity liable to degrade said oligosaccharide, said precursor and said intermediates;~~
- (ii) culturing said cell on a carbon-based substrate in the presence of at least one said exogenous precursor and lactose permease, under conditions inducing the internalization according to a mechanism of active transport of said exogenous precursor by said cell and the production of said oligosaccharide by said cell,

~~Wherein~~wherein the enzyme is a glycosyl-transferase selected from the group consisting of β -1,3-N-acetyl-glucosaminyl-transferase, β -1, 3-galactosyl-transferase, β -1, 3-N-acetyl-galactosaminyl-transferase, β -1, 3-glucuronosyl-transferase, β -1, 3-N-acetyl-galactosaminyl-transferase, β -1, 4-N-acetyl-galactosaminyl-transferase, β -1,4-galactosyl-transferase, α -1,3-galactosyl-transferase, α -1, 4-galactosyl-transferase, α -2, 3-sialyl-transferase, α -2, 6-sialyl-transferase, α -2, 8-sialyl-transferase, α -1, 2-fucosyl-transferase, α -1, 3-fucosyl-transferase and α -1, 4-fucosyl-transferase, and

wherein said culturing comprises:

(a) a first phase of exponential cell growth ensured by said carbon-based substrate;

(b) a second phase of cell growth limited by said carbon-based substrate which is added continuously; and

wherein said precursor is added during the second phase.